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Effect of Progesterone, Mifepristone, and Estrogen Treatment During Early Pregnancy on Conceptus Development and Uterine Capacity in Swine[1]

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ABSTRACT

A series of experiments was performed to investigate the influence of progesterone at Days 2 and 3 of pregnancy on conceptus development and uterine capacity. In experiment 1, unilaterally hysterectomized-ovariectomized (UHO) white crossbred gilts were given no treatment, estradiol valerate (5 mg given on Days 11 and 12), or progesterone (200 mg/day on Days 2 and 3 after mating). On Day 105 of pregnancy, each fetus and its associated placenta were weighed, and the number of live and dead fetuses was recorded for each litter. Early progesterone treatment reduced ($P < 0.05$) litter size (a measure of uterine capacity in UHO gilts). In experiment 2, intact white crossbred gilts were mated, given no treatment or progesterone treatment on Days 2 and 3 of pregnancy, and farrowed. Progesterone treatment decreased ($P < 0.05$) pregnancy rates. In pregnant gilts, progesterone had no effect on the number of live or stillborn piglets at birth, and gestation length was decreased ($P < 0.05$). Progesterone treatment did not affect the number of large or small piglets. In experiment 3, intact gilts were mated at estrus and then received 1) no treatment or treatment with 2) 100 mg, 3) 200 mg, or 4) 400 mg mifepristone (also known as RU486) on Day 2 of pregnancy. On Day 11 of pregnancy, both uterine horns were flushed, the number and diameter of each conceptus was recorded, and the flushed material was assayed for total protein and acid phosphatase. The 400 mg mifepristone treatment decreased conceptus diameter ($P < 0.05$) and total protein ($P = 0.06$) in the uterine flushings. In experiment 4, UHO gilts were mated at estrus, injected with either corn oil (control) or mifepristone (400 mg) on Day 2 of pregnancy, and killed on Day 105 of pregnancy, and the number and weight of live fetuses and placentas was recorded. In contrast to the effect of progesterone treatment, mifepristone decreased uterine capacity by decreasing the number of small conceptuses. These data suggest that progesterone concentrations on Days 2 and 3 of pregnancy in swine influence the rate of conceptus development during early pregnancy and uterine capacity during later pregnancy.

embryo, estradiol, placenta, pregnancy, progesterone

INTRODUCTION

In the Meishan swine breed, which has greater fertility than European breeds, uterine protein secretion [1] and conceptus development and conceptus estrogen secretion [1–3] during early pregnancy are reduced compared with European breeds. One hypothesis is that this slower conceptus development during early pregnancy results in smaller placentas during later pregnancy and allows the uterus to accommodate more fetuses, thus increasing uterine capacity [3, 4], which is defined as the number of live fetuses that can be maintained by the uterus during gestation.

Previous experiments [1] also indicated that treatment of pregnant white crossbred gilts with progesterone on Days 2 and 3 of pregnancy accelerated the normal changes in uterine protein secretion occurring at the time of maternal recognition of pregnancy (Day 11). Conceptus estrogen secretion also was increased on Day 11 after early progesterone treatment, which suggested that conceptus development was accelerated. Thus, because early progesterone treatment had effects opposite to those present in the Meishan breed, we hypothesized that this treatment may increase the size of the conceptus during later gestation and decrease uterine capacity and litter size.

The slower rate of conceptus development in Meishan gilts has been suggested to be due in part to decreased conceptus estrogen secretion in this breed [3]. However, others have suggested that both the decreased estrogen secretion and the slower growth rate are a consequence of the decreased uterine protein secretion of the Meishan [1]. In support of the hypothesis that the rate of conceptus development in the Meishan is due to conceptus estrogen secretion, estrogen treatment of Meishan gilts on Days 12 and 13 of pregnancy significantly increased placental weights. This increase in placental weights was not associated with a decrease in litter size [5]. However, the number of observations in this experiment may have precluded obtaining a significant association between the two traits. It remains uncertain what effect estrogen treatment might have on placental weights and uterine capacity in European breed gilts.

Mifepristone (also known as RU486) has been reported to be a progesterone and glucocorticoid receptor antagonist [6, 7]. This activity forms the basis of the use of mifepristone as a contraceptive [8]. A single dose of mifepristone of sufficient magnitude abrogates pregnancy in humans. However, its effects on pregnancy in swine have not been studied. The ability of mifepristone to interfere with the interaction of progesterone and its receptor offered the possibility that mifepristone might be used to delay the normal response of the uterus to progesterone during pregnancy. We predicted that this delay in the onset of progesterone effects on the uterus would delay both uterine protein secretion and conceptus development, given that early progesterone treatment accelerated both traits [1]. Such an effect would be similar to that in the Meishan breed, suggesting that mifepristone might be useful in improving uterine capacity and litter size.

The previously demonstrated effects of manipulation of progesterone and estrogen during pregnancy on the rate of conceptus development, combined with the hypothesis that

[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the U.S. Department of Agriculture.
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the Meishan accomplishes greater fertility by reducing the rate of conceptus development, suggested to us that manipulation of progesterone or estrogen during early pregnancy might influence uterine capacity and litter size. Although the effect of early progesterone on pregnancy success has been studied previously, no experiments on the influence of early progesterone specifically on uterine capacity have been performed. The unilateral hysterectomized-ovariectomized (UHO) surgical model was developed specifically for this purpose [9]. In this model, the number of viable embryos available typically exceeds the capacity of the remaining uterine horn, providing a measure of uterine capacity that is independent of ovulation rate, fertilization rate, and embryonic mortality. Thus, the objective of the first experiment was to test the effect of estrogen treatment and early progesterone treatment on uterine capacity in UHO gilts. The objective of the second experiment was to determine in intact gilts whether the accelerating effects of early progesterone treatment were still present at farrowing and whether progesterone influenced stillbirth rate or preweaning survival. The objective of the third experiment was to determine whether interference with progesterone using mifepristone would have effects in intact gilts opposite to those observed by Vallet et al. [1]. Experiment four tested the effect of decelerating conceptus development with mifepristone on uterine capacity using UHO gilts. Thus, the primary objectives of the following experiments were to test the effect of progesterone, mifepristone, or estrogen treatment on conceptus development, uterine capacity, and litter size in European breed gilts.

MATERIALS AND METHODS

All experiments were performed according to Federation of Animal Science Society guidelines for the use of agricultural animals in research, and each experiment was reviewed and approved by the MARC Institutional Animal Care and Use Committee.

Experiment 1

Intact white crossbred gilts were unilaterally hysterectomized-ovariectomized at approximately 160 days of age. At 200 days of age, these gilts were observed once daily for estrous behavior and were mated after at least one estrous cycle of normal length (17–23 days) using artificial insemination with semen collected from mature white crossbred boars. Gilts were then randomly assigned to receive 1) no treatment (control), 2) estradiol valerate treatment (5 mg estradiol valerate in corn oil given on Days 11 and 12 of pregnancy), or 3) progesterone treatment (200 mg/day progesterone in corn oil given on Days 2 and 3 of pregnancy). At approximately 105 (103–106) days of pregnancy, gilts were killed, and the remaining gravid uterine horn was recovered and opened. A blood sample was collected from each live fetus, each fetus was separated from its placenta and weighed, and each placenta was dissected from the endometrium and weighed. The total number of live (determined by the presence of a heartbeat) and dead fetuses and the number of corpora lutea (CL) were recorded for each gilt.

Experiment 2

Intact white crossbred gilts were observed once daily for estrous behavior beginning at 200 days of age and then were mated at standing estrus by artificial insemination using semen collected from mature white crossbred boars. Gilts were randomly allocated to receive either no treatment (control) or 200 mg/day of progesterone on Days 2 and 3 of pregnancy. Gilts were farrowed, and gestation length was recorded for each gilt. At birth, the numbers of live and stillborn piglets born to each gilt were recorded, and each piglet was weighed. Piglets were weighed again at weaning (approximately 21 days of age) and at approximately 56 days of age.

Experiment 3

Intact white crossbred gilts were checked once daily for estrous behavior and were mated by artificial insemination at standing estrus after at least one estrous cycle of normal length. Gilts were assigned at random to receive 1) no treatment or treatment with 2) 100 mg, 3) 200 mg, or 4) 400 mg mifepristone in corn oil on Day 2 of pregnancy (Day 0 = day of estrus). On Day 11, gilts were laparotomized, and each uterine horn was flushed with 20 ml sterile 0.9% saline. Pregnancy of each gilt was confirmed by the presence of conceptuses, and the number and diameter of conceptuses recovered were recorded for each gilt. Uterine flushings were evaluated for total protein [10] and total acid phosphatase [11].

Experiment 4

White crossbred gilts were unilaterally hysterectomized-ovariectomized at approximately 160 days of age, observed once daily for estrous behavior beginning about 200 days of age, and mated by artificial insemination at standing estrus after at least one estrous cycle of normal length. On Day 2 of pregnancy, gilts were assigned at random to receive either 1) corn oil (10 ml) or 2) 400 mg mifepristone in corn oil i.m. At 105 days of gestation, gilts were killed, and the reproductive tract of each gilt was recovered and opened. A blood sample for measurement of hematocrit was collected from each live fetus. The number of live and dead fetuses and the weight of each live fetus were recorded. Brain, heart, and liver weights for each fetus were also recorded. The placenta for each fetus was dissected from the endometrium and weighed.

Statistical Analysis

In experiment 1, differences in pregnancy rates between treatments were analyzed using χ^2 analysis. The number of CL and live fetuses for each gilt were analyzed by ANOVA using a model that included the effect of treatment. Placental weights, fetal weights, and fetal hematocrits were averaged for each litter, and the litter averages were then analyzed using ANOVA with a model that included the effect of treatment. For fetal weights, average placental weight for that gilt was used as a covariate in the analysis. To assess whether treatment effects on uterine capacity were uniformly distributed among conceptuses of different weights, the number of fetuses weighing more than (large) and less than (small) the mean fetal weight of the control group and the number of placentas weighing more than (large) and less than (small) the mean placental weight of the control group were determined for each gilt. The effects of treatments on the number of large and small fetuses and placentas in each litter were then analyzed by ANOVA. To more fully evaluate treatment effects, the following contrasts were used: 1) no-treatment controls versus estradiol valerate treatment (effect of estradiol) and 2) no-treatment controls versus progesterone treatment (effect of progesterone).

In experiment 2, pregnancy rates were analyzed using χ^2 analysis. Gestation length, the number of liveborn and stillborn piglets, and the number of live and dead piglets at weaning for each gilt were analyzed by ANOVA using a model that included the effect of treatment. Piglet weights at birth, weaning, and Day 56 of age were averaged for each litter, and the litter averages were analyzed by ANOVA using a model that included the effect of treatment. Gestation length and litter size at birth were also used as covariates in the analysis of birth weights. For weaning and Day-56 weights, the true age of each litter when measurements were recorded was used as a covariate. In addition, the numbers of piglets weighing more than (large) and less than (small) the mean piglet birth weight for the control gilts were determined for each litter, and the effect of treatment on the number of large and small piglets per litter was analyzed by ANOVA. Piglets were divided into birth weight classes ranging from >2250 g to <500 g (in 250-g increments), and the frequency of stillbirth and death by the time of weaning was calculated for each weight class. The frequency of stillbirth and death before weaning for piglets was calculated for each gilt, and these frequency data were analyzed using an ANOVA with the effect of gilt and piglet size class as main effects.

In experiment 3, the average conceptus diameter and coefficient of variation (CV) was calculated for each litter. Six of 42 gilts (1 control, 3 treated with 100 mg mifepristone, and 2 treated with 200 mg mifepristone) had filamentous embryos, and the average conceptus diameter for these gilts was arbitrarily set to 10 mm (no CV could be calculated for these gilts). All other pregnant gilts contained spherical blastocysts. Average conceptus diameter, CV, total intrauterine protein, and total intrauterine acid phosphatase were analyzed by ANOVA using a model that included the effect of treatment. To more fully evaluate the effect of the different doses of mifepristone, each dose group was compared to the no-treatment control using separate nonorthogonal contrasts.

TABLE 1. Least squares means (±SEM) for traits from UHO gilts that received no treatment (control), estradiol valerate treatment on Days 11 and 12, or progesterone treatment on Days 2 and 3 of pregnancy and were killed at 105 days of gestation.

Trait	Control	Estradiol valerate	Progesterone
No. bred	30	23	35
No. pregnant	20	14	23
Uterine capacity (no. live fetuses)	6.9 ± 0.5	6.2 ± 0.6	5.6 ± 0.4[a]
No. fetuses > control mean fetal weight	3.5 ± 0.4	2.6 ± 0.5	3.0 ± 0.4
No. fetuses < control mean fetal weight	3.4 ± 0.5	3.6 ± 0.6	2.6 ± 0.5
No. placentas > control mean placental weight	3.2 ± 0.4	2.4 ± 0.5	2.3 ± 0.4
No. placentas < control mean placental weight	3.8 ± 0.6	3.8 ± 0.7	3.3 ± 0.5
No. mummies	0.8 ± 0.3	0.7 ± 0.4	1.3 ± 0.3
No. CL	14.6 ± 0.6	13.2 ± 0.8	13.3 ± 0.6
Placental weight (g)	203 ± 11	198 ± 13	201 ± 10
Fetal weight (g)	848 ± 21	840 ± 25	926 ± 19[b]
Hematocrit (%)	34.0 ± 0.6	34.4 ± 0.7	34.4 ± 5

[a] Progesterone group less than control ($P = 0.05$).
[b] Progesterone group greater than control after using placental weight as a covariate ($P < 0.01$).

In experiment 4, pregnancy rates between treatment groups were analyzed using χ^2. Number of CL, litter size, average fetal hematocrit, average fetal weight, average brain weight, average heart weight, average liver weight, and average placental weight (each trait averaged within litter) were analyzed using ANOVA with a model that included the effect of treatment. Average brain weight, heart weight, and liver weight were also subjected to ANOVA using average fetal weight as a covariate and a model that included the effect of treatment. The numbers of large (more than the control mean fetal weight) and small (less than the control mean fetal weight) fetuses and the number of large (more than the control mean placental weight) and small (less than the control mean placental weight) placentas were determined for each litter. The effect of mifepristone on the number of large and small fetuses and placentas was analyzed by ANOVA as described for experiment 1. Regression analysis was used to define the relationships between fetal weight and fetal brain, heart, and liver weights. For each trait, the gilt by fetal weight or gilt by fetal weight squared interaction, depending on the order of the relationship (linear or quadratic), was used as the error term.

RESULTS

Experiment 1

Pregnancy rate was 65% for gilts in this experiment and did not differ among treatments (Table 1). No significant differences were observed between control and estradiol valerate-treated gilts for any of the other traits measured in this experiment. The number of live conceptuses was decreased ($P = 0.05$) for progesterone-treated gilts compared with control gilts. Although the number of dead fetuses was numerically greater in the progesterone-treated gilts, the effect of progesterone treatment on the number of dead fetuses (mummies) was not significant. Placental weights and fetal hematocrits did not differ between progesterone-treated and control gilts. In contrast, fetal weights, after fitting placental weight as a covariate, were increased ($P < 0.01$) in the progesterone-treated gilts compared with control gilts. No differences were observed in the number of large and small fetuses or placentas between control and progesterone-treated gilts.

Experiment 2

Pregnancy rates were 75% and 58% ($P < 0.05$) for control and progesterone-treated gilts, respectively (Table 2). For pregnant gilts, progesterone treatment had no effect on the number of piglets born alive or stillborn or the number weaned alive or dead at weaning (Table 2). Piglet birth weight, weaning weight, and Day-56 weight was also not affected by progesterone treatment. Gestation length was 0.5 days shorter ($P = 0.05$) in progesterone-treated gilts compared with control gilts. Similar to experiment 1, progesterone treatment had no detectable effect on the number of large or small piglets.

Frequencies of piglet stillbirths and death by the time of weaning are illustrated in Figure 1. The incidence of both stillbirths ($P < 0.05$) and loss before weaning ($P < 0.01$) increased if piglets weighed <1 kg at birth.

Experiment 3

Results of this experiment are summarized in Table 3. Mifepristone appeared to have a biphasic effect. Conceptus diameter ($P = 0.07$) and uterine acid phosphatase secretion ($P < 0.05$) were both increased at the 100-mg dose. By

TABLE 2. Least squares means (±SEM) for traits from intact gilts that received no treatment (control) or were treated with progesterone on Days 2 and 3 of pregnancy.

Trait	Control	Progesterone
No. bred	104	58
No. farrowed	78	34
Gestation length (days)	115.97 ± 0.14	115.47 ± 0.21[a]
No. piglets born alive	9.4 ± 0.3	8.8 ± 0.5
No. piglets stillborn	0.78 ± 0.13	0.68 ± 0.19
No. piglets weaned	8.3 ± 0.3	8.0 ± 0.5
No. piglets dead at weaning	1.13 ± 0.15	0.97 ± 0.23
No. piglets > control mean weight at birth	5.4 ± 0.3	5.5 ± 0.5
No. piglets < control mean weight at birth	4.8 ± 0.4	3.8 ± 0.6
Piglet birth weight (kg)	1.58 ± 0.02	1.58 ± 0.03
Piglet Day-21 weight (kg)	5.13 ± 0.10	5.44 ± 0.15
Piglet Day-56 weight (kg)	16.7 ± 0.28	16.7 ± 0.43

[a] Progesterone group less than control ($P < 0.05$).

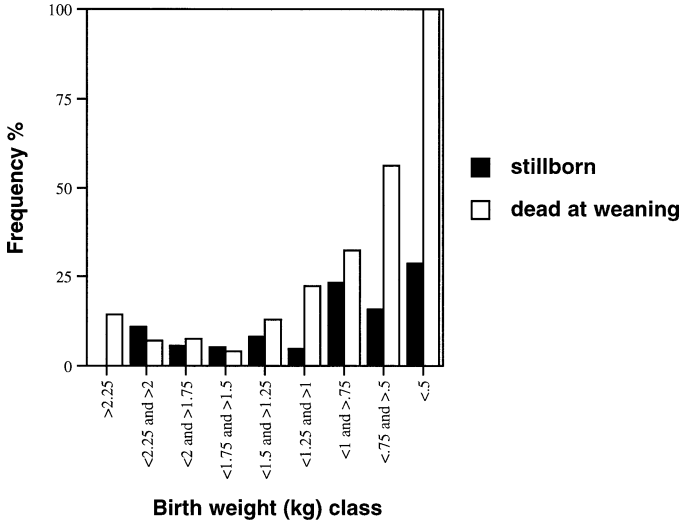

FIG. 1. The frequency of stillborn piglets and piglets that did not survive until weaning within different birth-weight classes. The chances that a piglet would be stillborn ($P < 0.05$) or would die before weaning ($P < 0.01$) increased when birth weights were <1 kg compared with piglets weighing >1 kg at birth. All piglets weighing <0.5 kg at birth died before weaning.

TABLE 4. Least squares means (±SEM) for traits from UHO gilts treated with either corn oil (control) or mifepristone on Day 2 of pregnancy.

Trait	Control	Mifepristone
No. bred	47	44
No. pregnant	38	34
Uterine capacity (no. live fetuses)	7.3 ± 0.3	4.7 ± 0.4[a]
No. fetuses > control mean fetal weight	3.6 ± 0.3	2.9 ± 0.3
No. fetuses < control mean fetal weight	3.7 ± 0.4	1.8 ± 0.4[a]
No. placentas > control mean placental weight	3.3 ± 0.3	2.5 ± 0.4
No. placentas < control mean placental weight	4.0 ± 0.4	2.2 ± 0.4[a]
No. mummies	0.8 ± 0.2	1.1 ± 0.2
No. CL	14.1 ± 0.4	14.2 ± 0.4
Placental weight (g)	204 ± 7	212 ± 8
Fetal weight (g)	859 ± 17	907 ± 18[b]
Fetal heart weight (g)	7.2 ± 0.2	7.6 ± 0.3
Fetal liver weight (g)	21.5 ± 0.8	23.9 ± 0.8[c]
Fetal brain weight (g)	26.3 ± 0.3	26.0 ± 0.3
Fetal hematocrit (%)	37.3 ± 0.5	37.1 ± 0.5

[a] Mifepristone group less than control ($P < 0.01$).
[b] Mifepristone group greater than control after using placental weight as a covariate ($P = 0.05$).
[c] Mifepristone group greater than control ($P < 0.05$). Effect disappears after using fetal weight as a covariate.

contrast, conceptus diameter ($P < 0.05$) and total uterine protein secretion ($P = 0.06$) were decreased at the 400-mg dose. There was no effect of the four treatments on the number of conceptuses recovered on Day 11 of gestation. There was also no effect of the four treatments on variation in conceptus diameter, as measured by the average within-litter CV.

Experiment 4

Pregnancy rate in this experiment was 79% and did not differ between treatments (Table 4). Mifepristone clearly decreased uterine capacity at 105 days of gestation. The number of dead fetuses (mummies) at 105 days of gestation was numerically but not significantly greater in the mifepristone-treated UHO gilts than in control UHO gilts. The decrease in uterine capacity coincided with an increase ($P = 0.05$) in fetal weight (after using placental weight as a covariate). In contrast to progesterone treatment, analysis of the number of large and small fetuses and placentas indicated that the numbers of small fetuses and placentas were lower ($P < 0.01$) in the mifepristone-treated gilts compared with control gilts. The numbers of large fetuses and placentas were not affected by treatment. There was no effect of mifepristone treatment on overall placental weights, fetal heart and brain weights, or fetal hematocrit. Fetal liver weights were higher in mifepristone-treated gilts ($P < 0.05$) compared with control gilts, but this effect disappeared after fetal weight was used as a covariate, suggesting that the increase in liver weight essentially reflected the increase in fetal weight after mifepristone treatment.

Regression analysis of the fetal organ weight data indicated that heart and liver weights are linearly related to fetal weights: heart weight = 0.22 + 0.0082(fetal weight) (R^2 = 0.88), and liver weight = −0.86 + 0.026(fetal weight) (R^2 = 0.87). By contrast, the relationship between brain weights and fetal weights was curvilinear: brain weight = 16.74 + 0.017(fetal weight) − 0.0000061(fetal weight)^2 (R_2 = 0.42). A plot of this relationship (not shown) suggests that brain weights begin to decrease at <800 g fetal weight.

DISCUSSION

The first experiment demonstrated the effect of early progesterone treatment on uterine capacity in pigs. Uterine

TABLE 3. Least squares means (±SEM) for traits from gilts on Day 11 of gestation after treatment with 0–400 mg mifepristone on Day 2 of gestation.

	Mifepristone (mg)			
Trait	0	100	200	400
No. treated	11	10	11	10
No. pregnant	11	9	9	9
No. blastocysts	13.3 ± 1.5	13.0 ± 1.9	13.0 ± 1.8	14.0 ± 1.5
No. CL	15.3 ± 0.9	16.2 ± 1.0	15.5 ± 0.9	15.9 ± 1.0
Conceptus diameter (mm)	4.9 ± 0.7	7.0 ± 0.8[a]	6.4 ± 0.8	2.8 ± 0.8[b]
CV for conceptus diameter	0.19 ± 0.02	0.21 ± 0.03	0.17 ± 0.03	0.23 ± 0.03
Total protein (mg)	65 ± 9	73 ± 9	70 ± 9	45 ± 9[c]
Total acid phosphatase (units)	7.4 ± 7.5	34.2 ± 7.9[d]	15.1 ± 7.5	3.1 ± 7.9

[a] Greater than control after log transformation ($P = 0.07$).
[b] Less than control after log transformation ($P < 0.05$).
[c] Less than control after log transformation ($P = 0.06$).
[d] Greater than control after log transformation ($P < 0.05$).

capacity, measured as the number of live fetuses in UHO gilts, was decreased in progesterone-treated gilts compared with control gilts. This decrease occurred in association with increased fetal weights at 105 days of gestation. Further analysis suggested that the increased fetal weight was not due to differences in survival of large or small fetuses in progesterone-treated gilts. Thus, early progesterone treatment may have accelerated fetal development. In contrast, in the second experiment, early progesterone treatment did not influence litter size, stillbirth rate, or birth weights in intact pregnant gilts but did significantly shorten gestation length by approximately 0.5 days. This result again suggests that early progesterone treatment accelerated the normal course of pregnancy, causing earlier parturition. This acceleration occurred in intact gilts, with no effect on birth weight and no lasting effect on piglet growth rate after birth. These results led us to hypothesize that mifepristone, a progesterone receptor antagonist, would be useful in delaying conceptus development and uterine protein secretion, creating a uterine environment that increases uterine capacity and be similar to the oviductal and uterine environment of the Meishan breed [1]. Results of experiment 3 supported this hypothesis. For gilts treated with the 400-mg dose of mifepristone, both conceptus development and uterine protein secretion were lower in the mifepristone-treated gilts compared with controls. Because these results are qualitatively similar to previous results obtained using Meishan gilts, the effect of this treatment on uterine capacity was examined. Unexpectedly, uterine capacity was also decreased in the mifepristone-treated gilts compared with controls. Unlike the effect of early progesterone treatment, mifepristone resulted in fewer small conceptuses (both small fetuses and small placentas) in the mifepristone-treated gilts. These results suggest that both too early a rise in progesterone and too late a rise in progesterone during early pregnancy are associated with decreased uterine capacity and that an optimum pattern of rise in progesterone concentrations likely exists during this period.

The 400-mg dose of mifepristone reduced both uterine protein secretion and conceptus size by about 50%, which is consistent with the hypothesis that progesterone concentrations during early pregnancy influence the timing of both of these events. However, the stimulatory effect of the 100-mg dose was unexpected and is more difficult to explain. Previous studies have demonstrated that both protein secretion [1, 12] and conceptus development [13] are highly variable and change rapidly at this stage of pregnancy (e.g., conceptuses change from spherical to filamentous blastocysts within 24 h). A factor contributing to this variation may be the use of once daily estrous detection in this experiment; the interval from first detection of estrus to ovulation in gilts varies from 23 to 48 h [14]. By chance, the gilts treated with 100 mg mifepristone may have been more advanced than the control gilts, resulting in three of the nine gilts already having filamentous blastocysts by Day 11. However, the statistical analysis indicated a P value of 0.07 for conceptus diameter and of <0.05 for acid phosphatase, suggesting that it is more likely that this stimulation is a real effect of a low dose of mifepristone. Many physiological explanations are possible, including potential effects on oviduct physiology, but without further information we can only speculate on the cause of the acceleration in conceptus development and protein secretion. Nevertheless, the 400-mg dose decelerated conceptus development and uterine protein secretion as expected, allowing a test of this treatment on uterine capacity.

The pattern of rise in progesterone during the early estrous cycle and pregnancy has been examined in numerous experiments [15–19]. Measurable increases in progesterone concentrations in the peripheral plasma are typically observed by Day 3, and luteal phase levels (15–25 ng/ml) are reached by about Day 6. The dose of progesterone used in the current experiment has also been used in numerous studies [20–25] to mimic progesterone concentration during the luteal phase. However, it is difficult to predict how much earlier the progesterone treatment used in this study triggers the timing mechanism for uterine protein secretion and conceptus development, primarily because the threshold for this trigger is not known. From the results of Vallet et al. [1], who used a treatment regimen identical to that used here, one can estimate an acceleration of about 24 h, which is consistent with the hypothesis that the low but measurable levels of progesterone present on Day 3 of the estrous cycle and pregnancy are sufficient to trigger progesterone timing. This finding is also consistent with the effect of mifepristone treatment on Day 2, because the half-life of this drug would prevent significant interference with progesterone beyond about Day 3. However, further studies are needed to establish the threshold concentration of progesterone necessary to trigger the progesterone timing mechanism.

Experiment 1, in which exogenous estradiol was administered, failed to demonstrate an increase in placental weights similar to that obtained previously using Meishan gilts [5]. Estrogen levels in the uterus of the Meishan from about Day 11 to Day 13 of gestation are significantly lower than those in the uterus of white crossbred gilts [1, 3]. The amount of estrogen already present in white crossbred gilts on Days 11 and 12 may be sufficient to saturate estrogen receptors, thus explaining the lack of effect of exogenous estrogen. An alternative test of the effect of estrogen during this period might be to determine the effect of an estrogen antagonist on placental weights and uterine capacity during later pregnancy. Unfortunately, many of the known estrogen antagonists (e.g., tamoxifen) have some estrogen agonist activity in swine [26, 27]. However, the effects of these and other estrogen antagonists on placental weight in mid-pregnancy have never been evaluated.

Results of the first and fourth experiments indicated that both progesterone and mifepristone treatment during very early pregnancy decreases the number of live fetuses in UHO gilts, suggesting that uterine capacity was decreased. However, this interpretation should be made with some caution. Other possible explanations for these results are that the treatments interfered with fertilization rate, oviductal transport, or early embryonic survival. Results of experiment 2 indicated a slight negative effect on overall pregnancy rate. However, for those gilts in which pregnancy was established, progesterone did not affect litter size, arguing against these mechanisms as an explanation for the results of experiment 1. In addition, results of experiment 3 indicate that the number of blastocysts was not reduced by Day 11 in gilts given doses of mifepristone up to 400 mg, suggesting that fertilization rate and oviductal transport were unaffected in mifepristone-treated gilts. Interference by mifepristone with the process of elongation, which occurs after Day 11, is still a possibility. However, decreased uterine capacity should have been reflected in an increased incidence of mummies, which would not occur if the explanation for the decrease in litter size was either failure of fertilization or decreased early embryonic survival due to failure or interference with elongation. Although the dif-

ference was not significant, the number of mummies was higher in both the progesterone- and the mifepristone-treated gilts compared with control gilts, consistent with at least a portion of the differences in litter size between control gilts and progesterone- or mifepristone-treated gilts being due to decreased uterine capacity. It is currently unclear whether all fetuses that die after Day 30 of gestation (those lost due to uterine capacity) remain present in the uterus as mummies for the duration of gestation.

Both progesterone and mifepristone treatment increased average fetal weights. However, progesterone treatment did not affect the number of large or small fetuses or placentas. In contrast, mifepristone specifically reduced the number of small fetuses and placentas but had no effect on the number of large fetuses or placentas. These results suggest that progesterone likely acted by accelerating fetal growth. The faster growth rate may have increased the risk of loss due to limitations in uterine capacity. Mifepristone treatment, by contrast, may have decreased the survival rate of smaller fetuses in some way, causing them to be underrepresented in the litter and thus decreasing litter size. Decreased progesterone during early pregnancy might influence many aspects of either uterine or conceptus physiology, resulting in the preferential loss of smaller conceptuses. Changes in uterine protein secretion, uterine blood flow, conceptus elongation, placental development, or aspects of fetal function are all possible mechanisms.

A high plane of nutrition has been reported to impair embryonic survival [28, 29], and this effect may be mediated by low progesterone concentration during the first few days of pregnancy [30–32]. Progesterone treatment during early pregnancy has been suggested as a way to alleviate this effect, improve embryonic survival, and thus improve litter size. However, experiments in which the alleviation of nutritional effects by progesterone treatment was attempted have resulted in equivocal results [33]. The current results could suggest the hypothesis that giving exogenous progesterone to compensate for the effect of nutrition is extremely difficult because it would result in many cases in either too much progesterone or inappropriate timing of progesterone influence and would therefore also result in decreased uterine capacity and litter size. To properly compensate for the effect of nutrition on progesterone, the optimum rate of increase of progesterone during early pregnancy must first be defined; this optimum has not been established.

Mifepristone treatment had no apparent effects on heart or brain weights, and the effect on liver weights could be explained by the effect of the drug on small fetuses. Overall, regression analysis indicated that brain weights were much less affected by reductions in fetal size than were liver and heart weights, which is consistent with the findings of others [34, 35]. The relationship between brain weight and fetal weight is quadratic, which confirms that mechanisms exist that spare brain development when nutrients are limited. These mechanisms appear to fail below a fetal weight of 800 g. Although numerous other factors also contribute to the loss of small piglets [36, 37], improper brain development that occurs in these small fetuses may contribute to perinatal and neonatal losses.

These data indicate that early progesterone treatment decreased uterine capacity at 105 days of gestation in UHO gilts, possibly by accelerating fetal growth. Early progesterone treatment had limited effects on litter size and birth weights in intact white crossbred gilts. Mifepristone treatment on Day 2 of gestation decreased both conceptus diameter and uterine protein secretion measured on Day 11. Surprisingly, mifepristone treatment on Day 2 also decreased uterine capacity measured on Day 105. The decrease in uterine capacity in response to mifepristone treatment apparently resulted from a decrease in the number of small (less than the control mean for fetal weight) fetuses at 105 days of gestation; the number of large fetuses was unaffected. These results suggest that both too much and too little progesterone during the first 2 or 3 days of pregnancy have a detrimental effect on uterine capacity. Efforts to define the optimum naturally occurring rate of progesterone rise combined with genetic selection to optimize this trait in pig populations could lead to increased uterine capacity and litter size.

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